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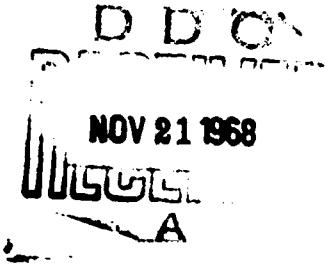

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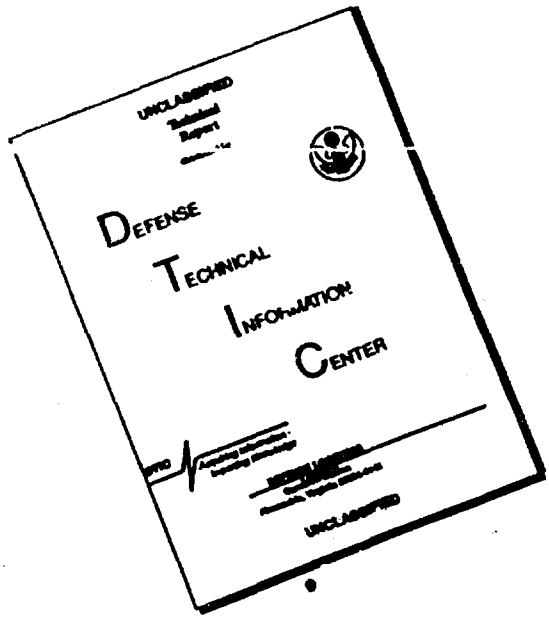

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THE DIAGNOSIS OF COTTON PLANT RESISTANCE TO VERTICILLIUM WILT

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Trudy Vsesoyuznogo Nauchno-Issledovatel'skogo Instituta Zashchity Rasteniy (Works of the All-Union Scientific Research Institute of Plant Protection), No 26, 1966, pp 125-131.

The most radical method of controlling the Verticillium wilt of cotton is the development of its resistant varieties. However, the selection of resistant forms of cotton against the infectious background is labor-consuming and little efficient. Considering that this evaluation should consitute the final stage of the work, we have set ourselves the task to develop accelerated methods for the primary evaluation of resistance of the cotton plant to wilt.

To solve this question there has been undertaken a study of the resistance of cotton to wilt in plant ontogeny and of peculiarities of the propagation of infection in leaves.

It was considered that the infection of cotton with wilt disease occurs during the period of the formation of lateral radicles at the stage of 3-5 true leaves, with its manifestation occurring during the period of budding. We have proved by direct experiments the possibility of an early infection of cotton at the cotyledon stage, at the 1st, 2nd, and 3rd true leaves.

The investigations have established that under natural conditions the fungus which is the causative agent of verticilliaceous wilt can be assured of good sources of nutrition directly in the seed hole itself. The nutrient substrate is formed by ungerminated seeds, parts of plants left in the soil during thinning, partly gnawed by boll worm, and plants that perished due to the

recurrence of cold weather. All these vegetable remnants contribute to an intensive development of the fungus in the seed hole and may bring about an early infection of the cotton plant with wilt [1]. This set of circumstances was confirmed by investigations of the UzbIZR [Uzbek Institute of Plant Protection] and SamGU [Samarkand State University] at the Symposium on Wilt in 1963.

The presence of the pathogen of wilt in the leaves is of great importance in an accelerated evaluation of the wilt resistance of cotton plant.

The ability of the parasite to penetrate into the leaves was used by us to ascertain the possibility of evaluating the wilt resistance of the cotton plant through the inoculation of leaves separated from the plant. The cotton plants in the field were infected by highly virulent strains of the pathogen of verticilliaceous wilt.

To diagnose the infection the use was made of the macroluminescent method. In the veins of leaves of plants affected with verticilliaceous wilt and examined in a flux of ultraviolet rays there is observed a so-called primary luminescence, i. e. natural luminescence of veins. As a source of ultraviolet rays the use can be made of a SVDSh-120A mercury-quartz lamp with a UFS-3 filter, transmitting light waves within the range of 3600-4200 A. This part of the spectrum does not cause deep chemical changes in the investigated biological objects. The examination of leaves in a flux of ultraviolet rays takes place directly over a light filter in a shaded place at room temperature. The luminescence of veins of the infected cotton leaves is greenish-blue. In the control no such luminescence is observed. A checkup carried out at the Laboratory of Immunity of the VIZR [All-Union Institute of Plant Protection] of the correspondence between luminescence and presence of the wilt pathogen upon its determination by the biological method showed that in cotyledonal leaves the luminescence of veins coincides with the spread of the fungus in them. In the true leaves of plants of susceptible varieties this correspondence was also noted, whereas in plants of resistant varieties the luminescence outstrips the spread of the fungus.

The reliability of determination of the infection from the luminescence has been verified by us on 50 samples from the collection of the Central Asian Affiliate of the VIR [All-Union Scientific Research Institute of Plant Growing] among which there was a group of Soviet-selected varieties with known characteristics with regard to wilt, notably such varieties as 108-f and S-4727 (used as standards), 8196, 8517, S-1622, 152-f, 149-f, etc.

The majority of samples studied was represented by varieties

of American origin. They are of special interest because the selection of most of them was carried out purposely with regard to their resistance to wilt.

Table 1

Evaluation of Cotton Varieties with Respect to Their Resistance to Verticilliaceous Wilt by the Method of Inoculation of Plants in the Ontogeny

(1) Группа	№ по каталогу ВИР (2)	Сорт (5)	Оценка в фазе семядолей (8)				Оценка в фазе 2–3 настоящих листьев (13)			Оценка пораженности на инфекционном фоне (14)			
			(9) здоровых	(10) слабо пораженных	(11) средне пораженных	(12) сильно пораженных	слабо пораженных	средне пораженных	сильно пораженных	8/IX всего больных (15)	8/IX в сильной степени (16)	23/X всего больных	23/X в сильной степени
1	4878	Acala 44 WR	53	30	0	17	24	55	21	26	8	70	23
1	4868	Acala 4-42	55	28	17	0	100	0	0	20	0	72	42
1	4863	Coker 124c (17)	85	9	6	0	25	42	33	42	30	76	26
2	4875	Stoneville 7	35	33	16	16	5	45	50	60	22	59	29
2	4874	Stoneville 20	64	22	0	14	0	100	0	48	22	56	22
(3) Стандарт		108-ф (6)	17	0	33	50	8	17	75	50	42	65	41
3 (4) То же		С-4727 (7)	8	40	40	12	0	0	100	61	47	70	42

Key: 1--Group; 2--No. acc. to VIR catalog; 3--Standard; 4--ditto; 5--Variety; 6--108-f; 7--S-4727; 8--Evaluation at the cotyledon stage; 9--Healthy; 10--Slightly infected; 11--Medium infected; 12--Strongly infected; 13--Evaluation at the stage of 2-3 true leaves; 14--Evaluation of morbidity against infectious background; 15--Total of affected plants; 16--In a high degree; 17--Coker 124s

Notes: 1. Evaluation against infectious background on October 23 was carried out by Z.F.Belovaya; 2. A relatively low percentage of morbidity upon late evaluation of varieties 108-f and S-4727 is explained by the fact that they were sown against infectious background among wild forms of cotton resistant to wilt

The comparison of results of the evaluation of resistance of the samples (Table 1) by the method of inoculation of cotton plants at various stages of their development permits us to draw a conclusion relative to differences in the resistance of cotton to wilt in the ontogeny. Thereupon it was established that in accordance with specific changes in their resistance the cotton varieties can be divided into 3 groups.

1. Varieties comparatively weakly affected at all stages of their development and only at the end of vegetative period showing a high percentage of morbidity. The harmfulness of infection in these varieties is minimal since they succed in producing the crop. Among this group may be reckoned such varieties as Acala 44 WR, Acala 4-42, Coker 124s, Acala 1517s, etc.

2. Varieties which are being affected uniformly at all stages of their development with average degree of morbidity. Among this group of varieties can be reckoned, for example, Stoneville 20 and Stoneville 7.

3. Varieties which are being affected at all stages but which infrequently display at an early stage deviations towards the increase of morbidity. Among such varieties may be reckoned Deltapine, S-4727, and a number of others.

The above patterns of the character of infection of cotton with wilt, ascertained by us on 50 samples, were confirmed in the subsequent investigation on 300 samples of various origin.

The obtained data point to the necessity of taking into consideration the differences in the infection of cotton with wilt at various stages of the development of plants while estimating the resistance of different selection samples as well as varieties produced by selectionists.

A more thorough study of the luminescence of veins during the infection of cotton with wilt permitted us to ascertain the chemical nature of luminescing substances. Biochemical investigations have established that in response to the infection there is activated in the resistant varieties of plants the formation of caffeic acid which is highly toxic to the pathogen of verticilliaceous wilt. The luminescence assumes a greenish-yellow hue due to scopoline compound.

The use of this method affords the possibility, while dealing with individual leaves, to rapidly diagnose the infection before the external symptoms of morbidity become apparent.

Summing up briefly the data relating to the diagnosis of the resistance of cotton to wilt with respect to phenol compounds we may state that: 1) the resistance of a variety is characterized by its ability to form upon infection the phenolic substances which are toxic for wilt pathogen; 2) in over-all determination of phenols by quantitative methods among uninfected plants the first place as to the amount of phenols is occupied by susceptible varieties, but after the infection the picture changes, indicating the ability of resistant varieties to activate phenols in response to the infection.

The study of physiological properties of cotton varieties has demonstrated a varying intensity of exosmosis processes in the cells.

The permeability, as a physiological index of resistance, has been studied on a number of cultures in various diseases [2, 3]. It was established that exosmosis in resistant plant varieties is considerably weaker than in the susceptible plant varieties. This circumstance has been ascertained by us also with regard to verticilliaceous wilt of the cotton plant with the use of various methods of determination of the degree of permeability of the protoplasm: by direct titration of leaf infusions with 0.0025 N $KMnO_4$, back titration with oxalic acid (0.05 N), and according to the index of refraction scale of a ITR-1 device. The method of direct titration can be used directly under field conditions, while the method of back titration is more suited for laboratory conditions. The results of determination of the permeability of cells of the cotton plant by the above-indicated methods are given in Table 2.

Table 2

Permeability of the Protoplasm in Various Cotton Varieties Differing in Their Resistance to Wilt (at the stage of cotyledon)

№ по каталогу ВИР (1)	Сорт, линия (3)	Количество экзосмированных органических веществ (7)		
		при титровании (8)		по шкале преломления рефрактометра ИТР-1 (11)
		прямом (9)	обратном (10)	
4878	Acala 44	0,10	0,10	106,6
4421	Acala 4-42 (4)	0,25	0,10	105,7
4613	Acala 1517c	0,25	0,30	108,4
5039	Deltapine (5)	1,00	1,35	119,7
(2) СТ	108-ф	0,50	0,60	114,0
СТ	С-4727 (6)	1,10	1,00	—

Key: 1--No. acc. to VIR catalog; 2--ST; 3--Variety, line; 4--Acala 1517s; 5--108f; 6--S-4727; 7--Number of exosmosed organic substances; 8--Upon titration; 9-Direct; 10--Back; 11--Acc. to refraction scale of ITR-1 device.

Determination of the resistance of varieties and lines of the cotton plant according to the number of substances exosmosed by the cells was carried out using the following technique. With a drill (1 cm in diameter) there were cut 5 disks which were then

placed in the test tubes filled with 2 ml of water. The infusion lasted 30 min. After the removal of disks the infusions were submitted to a direct titration with 0.0025 N $KMnO_4$ solution until the appearance of a stable pink color.

To obtain more distinct differences the method of back titration is used. For this purpose 20 disks are being placed in the flasks filled with 10 ml of distilled water. The time of infusion is increased to 2 hours. Then 5 ml of infusion is withdrawn with a pipet from each flask and put into clean flasks, adding 10 ml of fourfold diluted H_2SO_4 and 5 ml of 0.05 N $KMnO_4$. The mixture is heated during one minute and then titrated with 0.05 N oxalic acid until decoloration.

While determining the permeability of cells with the aid of the IFR-1 device the infusions were prepared in the same way as when determining it by the method of back titration but afterwards the directions on the use of this device were followed.

Investigations carried out by us showed the possibility of determining the degree of the resistance of cotton varieties to wilt in the toxins of the fungus. The additional use of this method makes it possible to characterize more fully the resistance of analyzed samples.

Indications are available in the literature on the use of toxins of the fungus for characterizing the resistance of tomato to fusarial wilt [4] and of tobacco to verticilliaceous wilt [5].

In determining the resistance of the cotton plant to verticilliaceous wilt by the above-indicated method we were using the exotoxins of the pathogen in the filtrate of cultural liquid from the one-month old fungal culture (Capek's medium) and endotoxins in the form of an aqueous extract from a dry culture of Verticillium dahliae. The use of the latter procedure is more convenient while working under field conditions. The extract from the pathogen was prepared at the rate of 300 mg of dry culture per 10 ml of water at 90°. The plants at the cotyledonal stage or cotton leaves separated from the plant are placed in the filtrate or in aqueous solution of the dry culture of the fungus. The experience has shown that the work should be carried out under conditions of the maximum illumination. In cotton plants of resistant varieties the turgor is preserved for a longer time - for one hour and more, whereas in susceptible plants the rapid wilting occurs, beginning already after 1-15 min. The subsequent restoration of the turgor in plants after placing them back into water is an important index. The loss of turgor under the effect of toxins does not mean in all plants the loss of viability. In plants of resistant varieties there is observed the reversibility of the wilting process, whereas in susceptible plants, as a rule, the viability

is not restored. In the evaluation of intoxication we used a 4-grade scale: 0 - the preservation of complete turgor in fungal toxins; 1 - slight wilting, complete restoration of turgor in water; 2 - considerable wilting, weak restoration of turgor in water; 3 - irreversible wilting.

An evaluation with the use of the intoxication method affords an additional characterization of the degree of resistance but permits us to single out only the forms which are very contrasting as to their resistance.

A preliminary selection of wilt-resistant samples of the cotton plant may be performed after the infection of plants at cotyledonal stage and that of individual leaves, beginning from the 5th-7th true leaf, with the use of the macroluminescent method. For the diagnosis of the resistance we may also use the analysis of the quantitative composition of phenols in plants and their capacity to activate the formation of phenols in response to the infection.

The study of the wilt-resistance of cotton samples from the VIR collection by the methods of laboratory and field diagnosis permitted the separation of a number of forms interesting as to their resistance to verticilliaceous wilt. These are Indian samples - 216F (k-4738, k-320, k-4719), Buri (k-4730); Uganda samples - BP-50 (k-4966), A-2106 (k-4977); Argentinian samples - Mdenes (k-4608); Saenz Pena (k-4603); Central African sample - Reba (k-4855); Brazilian sample H-105 (k-4965); and a group of samples of American selection.

American samples may be considered as belonging to the Indian group, since Acala was introduced by Cook from India and underwent a further selection in the United States. From among the American samples of greatest interest as to their resistance are Acala 4-42 (k-4421), Acala 44 WR (k-5035), Acala 1517s (k-4867), Acala 44 (k-5033), and a number of others.

We give a comparative characterization of the morbidity of the introduced samples (Table 3).

The explanation of agroclimatic characteristics of those zones from which the more resistant to verticilliaceous wilt cotton samples are being included into our domestic collection has been verified by us with the use of the method of climatograms - climograms (graphs) represented in the form of two co-ordinate axes (on the axis of ordinates are given temperature data, on the axis of abscissas - the sum of monthly atmospheric precipitation)

The climograms were plotted for all the zones from which the cotton samples were entered into the collection. Their analysis

has shown that the centers which yield the material more resistant to verticilliaceous wilt are associated with warm and humid climatic conditions. At the same time, the climograms of these zones are

Table 3

Comparative Characteristics of Introduced Cotton Varieties with Respect to Their Resistance to Verticilliaceous Wilt

Происхождение (1)	Количество испытанных образцов (11)	Из них выделено (12)		
		слабо поражаемых (13)	средне поражаемых (14)	сильно поражаемых (15)
Индия (2)	24	6	18	0
Уганда (3)	3	2	1	0
Бразилия (4)	2	1	1	0
США (5)	60	14	36	10
Афганистан (6)	2	0	0	2
О. Тринидад (7)	8	0	5	3
Болгария (8)	39	0	25	14
Аргентина (9)	19	2	15	2
Греция (10)	18	0	11	7

Key: 1--Origin; 2--India; 3--Uganda; 4--Brazil; 5--United States; 6--Afghanistan; 7--Island of Trinidad; 8--Bulgaria; 9--Argentina; 10--Greece; 11--Number of samples tested; 12--Out of them were singled out; 13--Weakly affected; 14--Medium affected; 15--Strongly affected.

extended horizontalwards and are compact verticalwards. The annual temperature fluctuations are insignificant - not more than 4°, ranging from 24 to 28°. As an example we present the climograms of the zones of India [sic] - Singapore and Jakarta (Figure 1). A similar in character are climograms of the central regions of Africa, for example those of Mobaye and Luluabourg (Figure 2). The annual difference of temperatures in Mobaye is 4° and in Luluabourg - 0.5°. The average annual temperatures are 25.9 and 24.7°, respectively. The American Continent is represented by the climogram of Belize situated on the east coast of the Yucatan Peninsula. It is seen from the climograms quoted that they are all situated in the "warm" "humid" part of the drawing. The possibility of obtaining interesting forms from the central part of the American Continent is still enhanced by the fact that the latter is the homeland of the cotton plant cultivation.

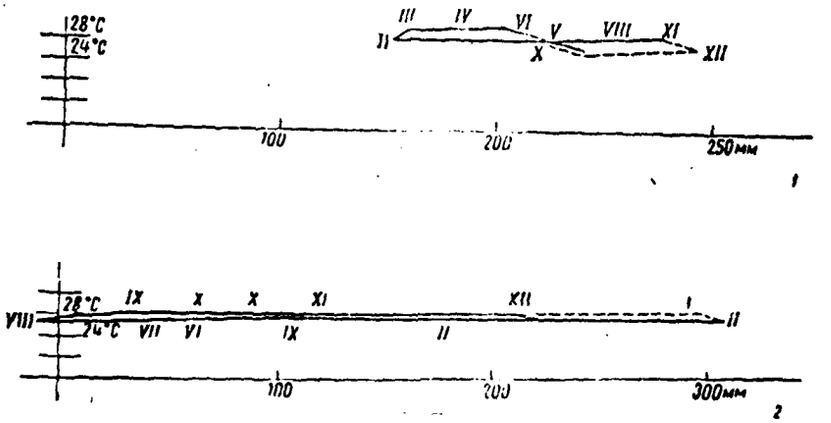

Fig. 1. Climograms of zones of India [sic]
1 - Singapore, 2 - Jakarta

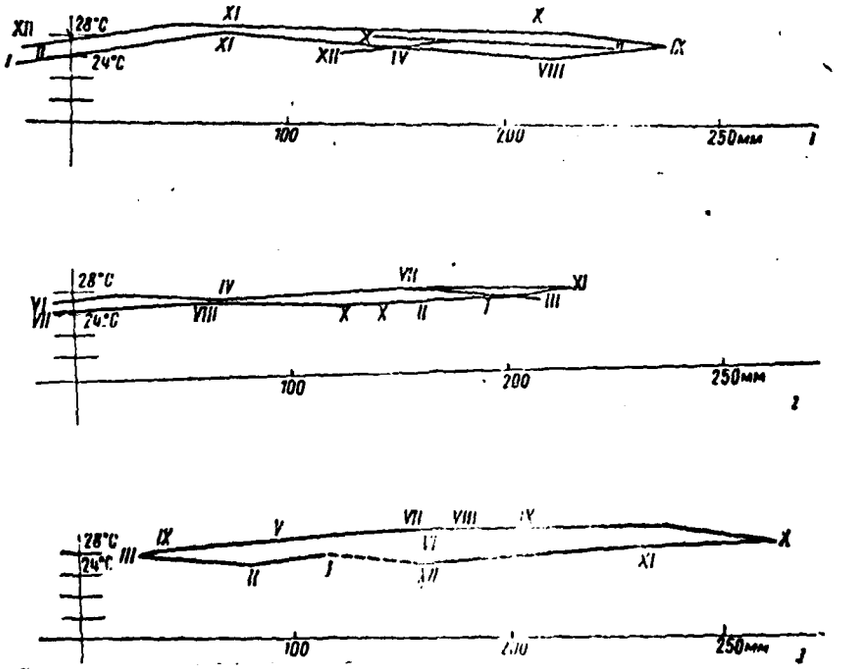

Fig. 2. Climograms of zones of Africa and America
1 - Mobaye, 2 - Luluabourg, 3 - Belize

However, the analysis of the possible incidence of this disease shows that these zones are favorable for the development of verticilliaceous wilt. Investigations have established that temperature optimum for the fungus is +22° and that the development of disease is especially intensive when it coincides with the presence of a relatively high moisture of soil.

Thus, the regions of India [sic], Uganda, and Central America are characterized by a climate which is favorable for the development of verticilliaceous wilt, and it is exactly in these zones that the natural selection of resistant material is possible. Therefore, it is from these very centers that we can obtain valuable samples for the selection of wilt-resistant cotton plants.

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